

A NEW POLYENE ANTIBIOTIC, FLAVOMYCOIN STRUCTURAL INVESTIGATIONS. II*

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The polyene antibiotic flavomycoin contains a pentaene chromophore which is conjugated with the lactone carbonyl group. This fact was proved by reduction of flavomycoin with lithium aluminum hydride, by low temperature ultraviolet spectroscopy and the infrared absorption of perhydro-flavomycoin. Oxidative degradation of perhydro-flavomycoin resulted in 2-methyl-tridecane-1,13-dioic acid which was identified by gas chromatography and mass spectrometry. A partial formula for flavomycoin is given.

Flavomycoin is a crystalline polyene antibiotic produced by *Streptomyces roseoflavus* ARAI 1951 var. *jenensis* nov. var. JA 5068. Its isolation and characterization are described in previous reports^{1,2)}. The molecular weight of 721 was determined by mass spectrometry. Elemental analysis and degradation of the molecule to the parent hydrocarbon led to the empirical formula $C_{41}H_{68}O_{10}$.

Flavomycoin was found to contain no methoxyl or acetoxy groups and does not react with periodate. C-Methyl determination gave a minimum value of 3 such groups. Further the presence of 5 double bonds, 8 hydroxyl groups and 1 lactone group was indicated. This paper reports the investigation of the chromophore of flavomycoin.

The yellow-green color and the broad absorption maximum at 363 nm of flavomycoin can only be explained by the fact that a polyenic system must be present. The maximum peak at 363 nm corresponds to the second peak of a hexaene³⁾. On the other hand we found by catalytic hydrogenation only 5 double bonds. Therefore it seemed possible that a pentaene system might be conjugated to a carbonyl function. To prove this supposition flavomycoin was reduced under several conditions.

At first flavomycoin was subjected to a treatment with sodium borohydride. The ultraviolet spectrum of the reduction product was identical to the spectrum of flavomycoin showing that in the chromophore no structural change had taken place. Then flavomycoin was reduced with lithium aluminum hydride dropping the solution of flavomycoin into a stirred lithium aluminum hydride suspension in tetrahydrofuran. The mixture was refluxed for 5 hours. The reduction product isolated by extraction with butanol showed an ultraviolet absorption spectrum with three bands

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at 318, 304 and 288 nm which corresponds to that of a typical tetraene³⁾.

This unexpected result can be explained, that under those conditions α, β -unsaturated lactones react with lithium aluminum hydride by simultaneous hydrogenation of the double bond, as is known from literature⁴⁾. Therefore we reduced flavomycoin under less drastic conditions, using the so-called inverted method⁵⁾. A solution of lithium aluminum hydride in tetrahydrofuran was dropped into a solution of flavomycoin dissolved in tetrahydrofuran-pyridine at -20°C , and the mixture was stirred for 10 hours. The isolated pale-yellow reduction product showed peaks at 355, 337 and 320 nm corresponding to those of pentaene antibiotics as presented in Fig. 1. This gave evidence that all 5 double bonds must belong to a pentaene system which is in conjugation to the lactone group.

This fact is also confirmed by a shift of the lactone carbonyl band in the infrared spectrum from 1705 cm^{-1} to 1730 cm^{-1} when flavomycoin is hydrogenated.

A direct method for determination of this novel chromophore type within polyene antibiotics we could accomplish by application of the low temperature ultraviolet spectroscopy. HAUSSER, KUHN *et al.*⁶⁾ had found that polyene carboxylic acids normally exhibiting a broad absorption peak, at low temperature *e. g.* -196°C , show a regular series of sharp peaks. Therefore the measurement of ultraviolet absorption of flavo-

Fig. 1. Ultraviolet absorption of LiAlH_4 -reduction product of flavomycoin (reduction temperature -20°C) and original flavomycoin.

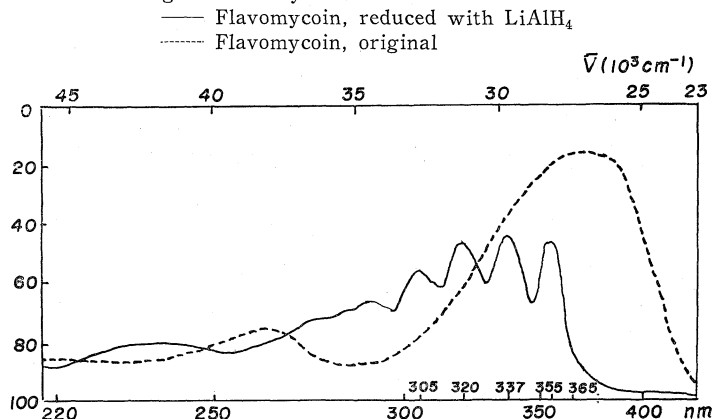


Fig. 2. Ultraviolet absorption of flavomycoin at -185°C and 25°C in methanol-ethanol.

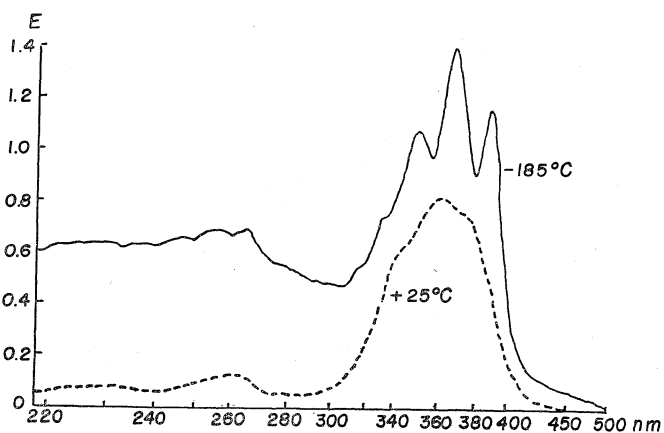


Table 1. Comparison of absorption bands of flavomycoin and unsaturated acids at low temperatures

Substance	Maxima (nm)		
	I	II	III
Flavomycoin ^{a)}	390	369	351
2, 4, 6-Octatrienoic acid ^{b)}	305	291	279
2, 4, 6, 8-Decatetraenoic acid ^{b)}	345	328	312
2, 4, 6, 8, 10-Dodecapentaenoic acid ^{b)}	384	363	344

a) At -185°C in methanol-ethanol

b) At -196°C in ethylether-ethanol⁶⁾

mycoin was carried out at -185°C . The resulting spectrum showed fine structure with maxima at 390, 369 and 351 nm as demonstrated in Fig. 2. Compared with the absorption behavior of some polyene carboxylic acids the ultraviolet spectrum of flavomycoin was found to be similar to that of dodecapentaenoic acid (Table 1). The shift of 6 nm to longer wave lengths can be explained by the fact that flavomycoin must be considered as a substituted derivative of this acid. Therefore in flavomycoin an analogue structure can be discussed.

The chromophore of flavomycoin must have all-*trans* configuration, because it is impossible to accomplish a *cis-trans* isomerization under conditions reported for trichomycin⁷⁾ and carotenoids⁹⁾. The existence of *trans* double bonds is explained by the distinctly marked band at 1010 cm^{-1} in the infrared spectrum of flavomycoin. According to LUNDE and ZECHMEISTER⁹⁾ *cis* double bonds absorb at $772\sim 778\text{ cm}^{-1}$. The weak band at 750 cm^{-1} in the infrared spectrum of flavomycoin cannot be attributed to an absorption of a *cis* double bond compared with the following fact: In the case of 8,8'-*cis*-crocetin-dimethylester the *cis*-peak appears at 775 cm^{-1} , whereas another peak at 747 cm^{-1} is present in the infrared spectrum of 8,8'-*cis*-crocetin as well as of all-*trans*-crocetin-dimethylester¹⁰⁾.

Since a *cis* double bond in flavomycoin is not detectable the "degradation" of the fine structure in the ultraviolet spectrum may be caused only by the conjugation of the lactone carbonyl group with the pentaene chromophore.

To decide whether the chromophore is branched or unbranched, the oxidative degradation of flavomycoin was accomplished by modification of methods of DJERASSI¹¹⁾ and CEDER.¹²⁾ Oxidative fusion of flavomycoin gave no higher dicarboxylic acids, but that of perhydro-flavomycoin gave 2-methyl-tridecane-1,13-dioic acid as largest dicarboxylic acid together with traces of 2-methyl-dodecane-1,12-dioic acid and short chain acids.

Flavomycoin was hydrogenated, the lactone group reduced by lithium aluminum hydride and the obtained polyol oxidized in acetic acid with chromic acid at 60°C . The dicarboxylic acid fraction was extracted from the reaction mixture and partly separated by preparative thin-layer chromatography. The identification of 2-methyl-

Fig. 3. Gaschromatogram of the purified dimethylester fraction (1% SE-30 on 140°C).

Mass spectra were taken of two components at the time which corresponds to the marks in the figure.

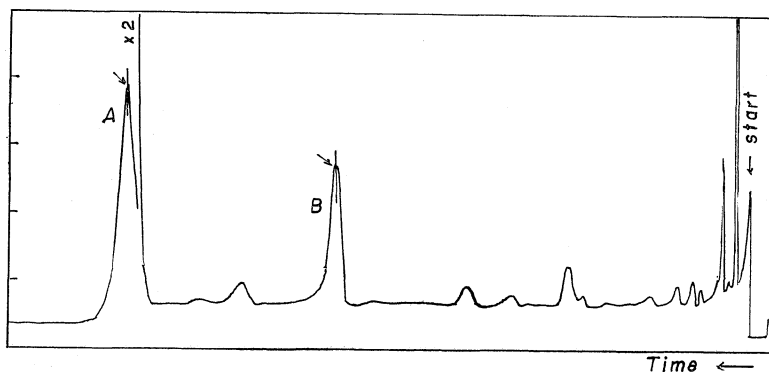
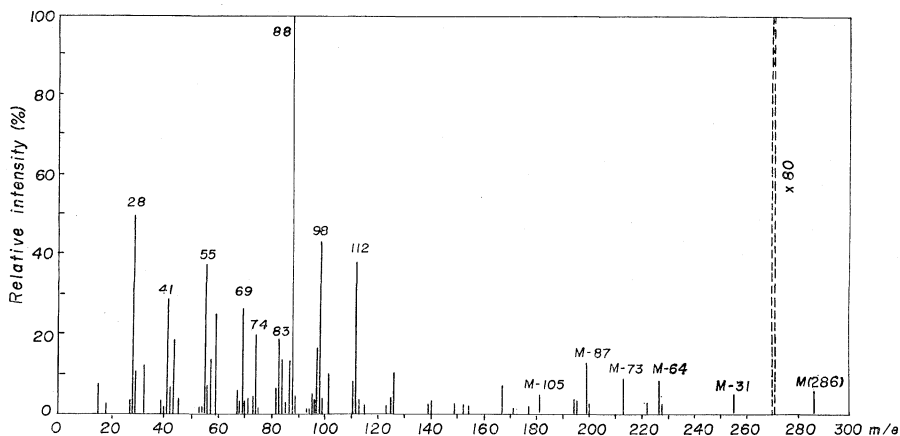


Fig. 4. Mass spectrum of the component A identified as dimethylester of 2-methyl-tridecanedioic acid.



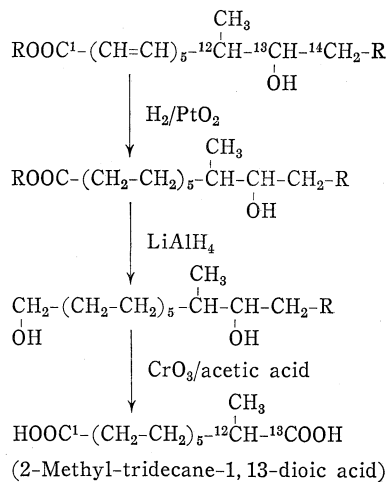
tridecane-1,13-dioic acid in form of dimethylester was carried out by gas chromatography, comparing the retention time with that of known acids on polar and unpolar columns, and by negative ion mass spectrography showing the parent peak at m/e 285 (M-1).

A very exact separation and identification of the dicarboxylic acids is possible by the combination of gas chromatography and mass spectrometry¹³⁾. The gas chromatographic separation by this method is shown in Fig. 3, the identification of the main peak (A) by mass spectrometry in Fig. 4. The highest peak at m/e 286 is the molecule ion peak (M⁺) of 2-methyl-tridecane-1,13-dioic acid dimethylester (calcd. 286.2).

The mass spectra of α -branched dicarboxylic acid esters have not yet been discussed in literature, but by utilizing the experience of RYHAGE and STENHAGEN¹⁴⁾ for straight chain analogs and esters of monomethyl-substituted long chain carboxylic acids, one can use the following approach. In unbranched methyl esters, one of principal cleavages occurs between the α and β carbon atoms to give m/e 74 and M-73, while in α -methyl esters the fragmentation occurs predominantly at the branched carbon to yield m/e 88 and M-87. In the case of dicarboxylic acid (A) the base peak at m/e 88 and the relative intensity of the peak at M-87 indicate clearly that this compound is the 2-methyl-tridecane-1,13-dioic acid dimethylester. The other peak (B) in the gas chromatogram was identified by these methods as 2-methyl-dodecane-1,12-dioic acid dimethylester.

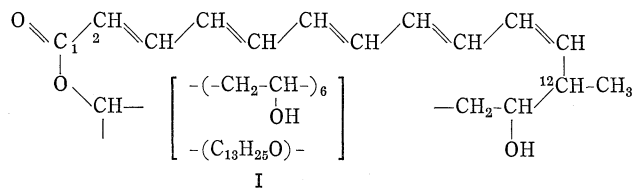
These results establish the structural features from C-1 to C-13 in flavomycoin and the presence of a hydroxyl group at C-13 (Fig. 5). Further there is a methyl group in this region which can be attached to C-12 or C-2. The final position of

Fig. 5. Degradation scheme of the chromophore of flavomycoin



the methyl group is to determine by ozonolysis, however according to the results obtained till now a position at C-12 is most probable.

In connection with earlier studies¹⁾ we can establish the following partial formula of flavomycoïn :



The results of our structural investigations on flavomycoïn point to a macrocyclic polyene structure. But in contrast to the most known pentaene antibiotics, flavomycoïn contains a pentaene system in conjugation with the lactone carbonyl group. The same chromophore type was found likewise in mycoticin¹⁶⁾ and later also in flavofungin¹⁵⁾. To a further structural difference between these antibiotics and flavomycoïn points the finding of an α -methyl-branched dicarboxylic acid by the oxidative degradation of perhydro-flavomycoïn, whereas the perhydro-compounds of mycoticin and flavofungin would give under similar conditions the unbranched tridecane-1, 13-dioic acid.

Experimental

Reduction of flavomycoïn with sodium borohydride

Flavomycoïn (0.1 g) was dissolved in 30 ml of 50 % ethanol, 0.02 g of sodium borohydride added and the mixture allowed to stand overnight. After neutralization at pH 6.8 and removal of ethanol the mixture was extracted with ethyl acetate, concentrated and precipitated by addition of ethyl ether. A yellow powder (0.08 g) was obtained which showed an ultraviolet spectrum being identical with that of the starting material.

Reduction of flavomycoïn with lithium aluminum hydride

a) at 65°C: One gram of flavomycoïn was dissolved in 30 ml of tetrahydrofuran-pyridine mixture (2:1) and added dropwise to 40 ml of tetrahydrofuran containing 0.8 mg lithium aluminum hydride. The mixture was stirred and refluxed for 10 hours. After decomposition of the unchanged hydride with water and acidifying to pH 5, tetrahydrofuran was evaporated, the residue diluted with water and extracted three times with 50 ml butanol. The extract was washed with 1 % sulfuric acid, water and 1 % sodium bicarbonate solution, evaporated to 25 ml and the pale-yellow reduction product precipitated with petroleum ether.

b) at -20°C: 0.53 g of flavomycoïn dissolved in a mixture of 60 ml tetrahydrofuran and 10 ml pyridine, was cooled to -40°C. After adding of 0.5 g lithium aluminum hydride dissolved in 20 ml of tetrahydrofuran, this mixture was stirred for 10 hours at an interior temperature of -20°C. The unchanged hydride was destroyed with water and the reduction product isolated as above described.

Oxidation of perhydro-flavomycoïn and identification of 2-methyl-1, 13-tridecanedioic acid

A sample of perhydro-flavomycoïn (2 g) was dissolved in 30 ml of tetrahydrofuran and the solution slowly added to a suspension of 4 g of lithium aluminum hydride and 80 ml tetrahydrofuran. The mixture was refluxed for 2 days, then the unchanged hydride destroyed with water and the mixture evaporated to dryness under reduced pressure. The inorganic material was dissolved in dilute acid at 0°C and the polyol was extracted with

butanol. The combined extracts were washed with water and 1% sodium bicarbonate and the butanol was removed under reduced pressure to give 1.8 g of an oily polyol.

The polyol (1.8 g) was oxidized with 10 g of chromium trioxide in 700 ml of glacial acetic acid at 65°C for 2 hours. The unchanged chromium trioxide was reduced by sulfur dioxide and the mixture was evaporated to dryness. The residue was dissolved in water and extracted with ethyl ether three times. The combined extracts were washed with a small amount of water, dried over sodium sulfate and evaporated to dryness; 1.1 g of a strong sour smelling oil were recovered. The short chain oxidation products were removed by preparative thin-layer chromatography (Silica Gel G; E. Merck, Darmstadt; layer: 1 mm; system: diisopropyl ether - formic acid - water (90:7:3), chamber saturation; length of run: 12 cm). The zone corresponding to dicarboxylic acids of C₁₀ to C₁₄ was eluted with methanol and the solution evaporated to dryness. The residue was dissolved in 30 ml of ethyl ether and esterified with diazomethane yielded 45 mg dimethyl ester fraction. The ester fraction was filtered over alumina and analyzed by gas chromatography at 153°C using a diethylene glycol succinate column. The gas chromatogram showed a number of peaks due to a series of homologous dimethyl esters. The compound of the largest peak (A) was identified by comparison of the retention time with test acids as 2-methyl-tridecanedioic acid. The negative ion mass spectrum of the ester fraction showed the highest molecule ion at m/e 285 (M-1), calculated for 2-methyl-tridecanedioic acid dimethyl ester 286.2.

For the combination gas chromatography-mass spectrometry¹³⁾ were used 1% SE-30 on Celite column and Atlas CH₄ mass spectrometer respectively. The components A and B (Fig. 3) were identified by direct mass spectrometry to be 2-methyl-tridecanedioic acid dimethyl ester (m/e=286) (Fig. 4) and 2-methyl-dodecanedioic acid dimethyl ester (m/e=272).

Isomerization attempts

a) 30 mg of flavomycoin were dissolved in a 10 ml mixture of acetone - acetic acid (1:1) at 60°C and allowed to stand 2 hours. Petroleum ether was added and after cooling the precipitate was collected by filtration, washed with acetone and ether and dried to yield a yellow powder. The ultraviolet absorption was unchanged.

b) According to BROWN & WALD¹⁷⁾, a methanolic solution of flavomycoin (0.1 mg/ml) and iodine (0.001 mg/ml) was irradiated for 2~40 minutes with white light of intensity of about 30 ft candles. No significant change of the ultraviolet spectra was observed.

Low temperature ultraviolet spectroscopy

The low temperature ultraviolet spectra were taken with a Beckman DK 2A using a quartz cell cooled with liquid nitrogen.

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